SAMPLE
Matrix: urine
Sample preparation: Filter (0.2 μm), inject an aliquot directly. Hydrolyze conjugates by heating with 6 M HCl at 37° for 18 h, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:1.5% trifluoroacetic acid on water 10:90
Flow rate: 0.5
Detector: UV or radioactivity

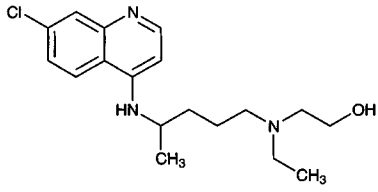
CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Extracted: phenol, phenyl glucuronide, phenyl sulfate

KEY WORDS
rat

REFERENCE
Hughes,M.F.; Hall,L.L. Disposition of phenol in rat after oral, dermal, intravenous, and intratracheal administration, *Xenobiotica*, **1995**, *25*, 873–883.

Hydroxychloroquine



Molecular formula: $C_{18}H_{26}ClN_3O$
Molecular weight: 335.88
CAS Registry No.: 118-42-3, 747-36-4 (sulfate)
Merck Index: 4863
Lednicer No.: 1 342

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5 M NaOH + 100 μL 10 μg/mL chloroquine in MeOH + 5 mL hexane:diethyl ether 50:50, vortex for 1 min, centrifuge at 1000 g for 10 min, freeze in dry ice/acetone, remove the organic layer. Thaw out the aqueous layer and repeat the extraction. Combine the organic layers and evaporate them to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm cyano (Regis)
Column: 75 × 4.6 3 μm Ultremex cyano (Phenomenex)
Mobile phase: 20 mM Dimethyloctylamine phosphate:60 mM ammonium acetate 40:60, pH adjusted to 4.5 (Dimethyloctylamine phosphate was prepared by adding phosphoric acid to N,N-dimethyloctylamine to precipitate the salt.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 320

CHROMATOGRAM
Retention time: 15
Internal standard: chloroquine (23)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Iredale,J.; Wainer,I.W. Determination of hydroxychloroquine and its major metabolites in plasma using sequential achiral-chiral high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *573*, 253–258.

SAMPLE
Matrix: blood
Sample preparation: Serum. 200 μL Serum + 50 μL 1 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Whole blood. 100 μL Whole blood + 500 μL water + 50 μL 1 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Dried blood. Spread 100 μL whole blood on a 70 × 30 mm piece of filter paper, allow to dry, cut paper into 10 × 5 mm strips, add 100 μL 1 μg/mL IS in water, add 1.5 mL 0.5 M NaOH, vortex for 30 s, let stand for 30 min at room temperature, add 300 μL MTBE, vortex for 30 s, centrifuge at 2000 g for 5 min, inject a 100 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 5 5 μm Spherisorb S5SCX sulfophenylpropyl-modified silica
Mobile phase: MeOH:water 98.5:1.5 containing 9.41 g/L ammonium perchlorate, adjust apparent pH to 8.0 with 220 mL/L 50 mM NaOH in MeOH
Flow rate: 1.5
Injection volume: 100
Detector: F ex 215 em no filter

CHROMATOGRAM
Retention time: 8
Internal standard: 6,8-dichloro-4-(1-methyl-4-diethylaminobutylamino)quinoline (5)
Limit of quantitation: 5 ng/mL (serum), 10 ng/mL (whole blood, dried blood)

OTHER SUBSTANCES
Extracted: chloroquine, quinine, metabolites
Simultaneous: acebutolol, N-acetylprocainamide, atenolol, butriptyline, chlorpromazine, desipramine, flecainide, fluoxetine, imipramine, labetalol, maprotiline, mepacrine, metoprolol, mexiletine, norbutriptyline, normaprotiline, procainamide, propranolol, sotalol
Noninterfering: amitriptyline, amodiaquin, carbamazepine, clomipramine, dapsone, diazepam, dothiepin, doxepin, fluvoxamine, lorazepam, mefloquine, nitrazepam, norclomipramine, nordiazepam, nordothiepin, nordoxepin, nortriptyline, primaquine, proguanil, pyrimethamine

KEY WORDS
serum; whole blood; dried blood

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. Simple and rapid HPLC of quinine, hydroxychloroquine, chloroquine, and desethylchloroquine in serum, whole blood, and filter paper-adsorbed dry blood, *J.Anal.Toxicol.*, **1994**, *18*, 255–260.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 2 mL 20 ng/mL propranolol hydrochloride in water, vortex for 10 s, sonicate for 10 min, centrifuge at 3000 rpm for 20 min. Remove 2 mL of the supernatant and add it to 1 mL 100 mM NaOH and 8 mL ethyl acetate:isopropanol 90:10, vortex for 30 s, centrifuge at 3000 rpm for 15 min. Remove the organic layer and evaporate it to dryness under a stream of air at 35-40°, reconstitute the residue in 250 μL MeOH, vortex for 30 s, inject a 10-100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 7 μm cyano (Applied Biosystems)
Column: 250 × 4.6 5 μm cyanopropyl (Baxter/Burdick & Jackson)

Mobile phase: MeCN:MeOH:buffer 50:30:20 (Buffer was 25 mM K_2HPO_4 adjusted to pH 6.0 with phosphoric acid.)
Column temperature: 50
Flow rate: 2
Injection volume: 10-100
Detector: F ex 230 em 385 (370 nm cut-off filter)

CHROMATOGRAM
Retention time: 9.72
Internal standard: propranolol (4)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood

REFERENCE
Wei,Y.; Nygard,G.A.; Khalil,S.K.W. A HPLC method for the separation and quantification of the enantiomers of hydroxychloroquine and its three major metabolites, *J.Liq.Chromatogr.*, **1994**, *17*, 3479–3490.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM
Retention time: 4.65
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.

Flow rate: 1

Injection volume: 20

Detector: UV 245, 256, 343

CHROMATOGRAM

Retention time: 11.93

Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)

Limit of detection: 2 ng/mL (343 nm)

OTHER SUBSTANCES

Extracted: betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluocinolone acetonide, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone

Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS

serum

REFERENCE

Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids, *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE

Matrix: blood, erythrocytes, urine

Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL buffer. Hemolyze erythrocytes in water 1:3. Dilute urine with water 1:99. Add 1 mL plasma, hemolyzed erythrocytes, or diluted urine to the SPE cartridge, wash with 4 mL buffer, wash with 2 mL MeOH:buffer 50:50, elute with 3 mL MeOH:ammonia 99:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in the initial mobile phase, vortex, inject a 50 μL aliquot. (Prepare buffer by mixing equal volumes of 100 mM ammonium formate and 100 mM ammonia solution, pH 9.2.)

HPLC VARIABLES

Guard column: 10 × 4 Inertsil
Column: 250 × 4 5 μm Inertsil
Mobile phase: Gradient. A was MeCN. B was MeOH:25% ammonia solution 92.5:7.5. A:B 78:22 for 3 min, then to 65:35 over 2 min (Waters curve no. 3), maintain at 65:35 for 20 min, return to 78:22 over 5 min (Waters curve no. 3).
Flow rate: 0.85
Injection volume: 50
Detector: F ex 325 em 375

CHROMATOGRAM

Retention time: 11.5
Internal standard: hydroxychloroquine

OTHER SUBSTANCES

Extracted: chloroquine, quinine, monodesethylchloroquine, bidesethylchloroquine
Simultaneous: halofantrine, quinidine
Noninterfering: proguanil, cycloguanil, 4-chlorophenylbiguanide, amodiaquine, mefloquine, pyrimethamine, sulfadoxine, cinchonine, cinchonidine

KEY WORDS

SPE; hydroxychloroquine is IS; plasma

REFERENCE

Chaulet,J.-F.; Robet,Y.; Prevosto,J.-M.; Soares,O.; Brazier,J.-L. Simultaneous determination of chloroquine and quinine in human biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 303–310.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: Chiral-AGP
Column: 150 × 4.6 Chiral-AGP (Regis)
Mobile phase: MeCN:EtOH:30 mM pH 7.0 sodium phosphate buffer 1:20:79 containing 5 mM N,N-dimethyloctylamine
Flow rate: 0.9
Injection volume: 50
Detector: UV 320

CHROMATOGRAM

Retention time: 10 (-), 14 (+)
Limit of detection: 5 ng

KEY WORDS

chiral

REFERENCE

Iredale,J.; Wainer,I.W. Determination of hydroxychloroquine and its major metabolites in plasma using sequential achiral-chiral high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *573*, 253–258.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm Chiral-AGP (ChromTech)
Mobile phase: MeCN:isopropanol:buffer 1:5:94 (Buffer was 50 mM $(NH_4)H_2PO_4$ containing 5 mM dihexylamine, pH adjusted to 7.0 with 3 M NaOH.)
Column temperature: 40
Flow rate: 1
Injection volume: 10-100
Detector: F ex 230 em 385 (370 nm cut-off filter)

CHROMATOGRAM
Retention time: 10 (S(+)), 13 (R(-))

KEY WORDS
chiral

REFERENCE
Wei,Y.; Nygard,G.A.; Khalil,S.K.W. A HPLC method for the separation and quantification of the enantiomers of hydroxychloroquine and its three major metabolites, *J.Liq.Chromatogr.*, **1994**, *17*, 3479–3490.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 5 Spherisorb S5SCX
Mobile phase: MeOH:water 98.5:1.5 containing 80 mM ammonium perchlorate, adjusted to pH 8.0 with 50 mM NaOH in MeOH
Flow rate: 1.5
Detector: F ex 215 no emission filter

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: hydroquinine, quinine, chloroquine

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, *693*, 289–306.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 9.60 (A), 3.23 (B)

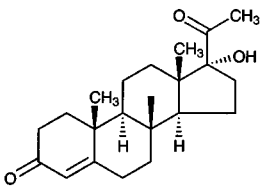
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine,

clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Hydroxyprogesterone



Molecular formula: $C_{21}H_{30}O_3$
Molecular weight: 330.47
CAS Registry No.: 68-96-2
Merck Index: 4886
Lednicer No.: 1 176

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 μg/mL dexamethasone in EtOH:water 10:90 + 100 μL 250 mM NaOH + 7 mL ether:dichloromethane 60:40, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 100 μL dichloromethane:EtOH:water 95:4:1, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Partisil silica
Mobile phase: Dichloromethane:EtOH:water 95:4:1
Flow rate: 1.5
Injection volume: 50
Detector: UV 239

CHROMATOGRAM
Retention time: 2.5 (17-hydroxyprogesterone)
Internal standard: dexamethasone (11.5)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, 11-deoxycortisol, hydrocortisone, 6α-methylprednisolone, prednisolone, prednisone, progesterone

KEY WORDS
plasma; normal phase

REFERENCE
Scott,N.R.; Chakraborty,J.; Marks,V. Determination of prednisolone, prednisone, and cortisol in human plasma by high-performance liquid chromatography, *Anal.Biochem.*, **1980**, *108*, 266–268.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeOH and water. Add 500 μL plasma to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeOH:water 20:80, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 1 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 25:25:50
Flow rate: 0.1
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 8 (17α-hydroxyprogesterone)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: androstenedione, 20α-hydroxy-4-pregnen-3-one, norethindrone, progesterone, testosterone

KEY WORDS
microbore; rat; plasma; SPE

REFERENCE
Taylor,R.B.; Kendle,K.E.; Reid,R.G.; Hung,C.T. Chromatography of progesterone and its major metabolites in rat plasma using microbore high-performance liquid chromatography columns with conventional injection and detection systems, *J.Chromatogr.*, **1987**, *385*, 383–392.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 500 μL water + 100 μL 10 μg/mL 3,7-dimethoxyflavone in EtOH + 8 mL diethyl ether, shake, centrifuge at 4° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:water 40:60, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 3 μm NS-Gel C18
Mobile phase: Gradient. MeOH:water from 40:60 to 55:45, maintain at 55:45 for 24 min, to 80:20 over 25 min
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: UV 210, UV 240

CHROMATOGRAM
Retention time: 36.52
Internal standard: 3,7-dimethoxyflavone (47)

OTHER SUBSTANCES
Extracted: aldosterone, androstenedione, dehydroepiandrosterone, deoxycorticosterone, 11-deoxycortisol, estradiol, estrone, hydrocortisone, pregnenolone, progesterone

KEY WORDS
serum

REFERENCE
Ueshiba,H.; Segawa,M.; Hayashi,T.; Miyachi,Y.; Irie,M. Serum profiles of steroid hormones in patients with Cushing's syndrome determined by a new HPLC/RIA method, *Clin.Chem.*, **1991**, *37*, 1329–1333.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 29.26 (17δ-hydroxyprogesterone)
Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES
Extracted: betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluendrenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids, *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE
Matrix: formulations
Sample preparation: Make up 1 mL injection to 100 mL with EtOH, remove a 1.2 mL aliquot and add it to 1 mL 1 mg/mL 17-hydroxyprogesterone in EtOH. Dilute this mixture to 50 mL with EtOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak CN
Mobile phase: MeOH:20 mM KH_2PO_4 40:60
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7 (hydroxyprogesterone caproate)
Internal standard: 17-hydroxyprogesterone (3)

OTHER SUBSTANCES
Simultaneous: benzyl benzoate
Noninterfering: polyethylene glycol 4000, mystristyl-gamma-picolinium chloride, methylcellulose, thimerosal

KEY WORDS
injections

REFERENCE
Das Gupta,V. Quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone by reversed-phase high-pressure liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 294–297.

SAMPLE
Matrix: formulations
Sample preparation: Injections. 1 mL Injection (200 μg/mL) + 300 mg NaCl + 200 μL 10% ammonia + 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 30 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot. Tablets. Weigh out amount of powdered tablets equivalent to about 200 μg compound, add 1 mL water, sonicate for 2 min, add 300 mg NaCl, add 200 μL 10% ammonia, add 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 30 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 3.5 acetate buffer 40:60 containing 1.5 mM triethylamine
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: 17α-hydroxyprogesterone

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, ergonovine, methylergonovine
Noninterfering: ascorbic acid

KEY WORDS
injections; tablets; 17α-hydroxyprogesterone is IS

REFERENCE
Tokunaga,H.; Kimura,T.; Kawamura,J. Determination of ergometrine maleate and methylergometrine maleate in pharmaceutical preparations by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1983**, *31*, 3988–3993.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets containing about 1 mg levothyroxine, add 4.5 mL 0.5 mg/mL hydroxyprogesterone caproate in MeOH, add 20.5 mL 10 mM NaOH in MeOH:water 75:25, shake intermittently for 5 min, filter, discard first 5 mL filtrate, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak CN

Mobile phase: MeCN:0.1% phosphoric acid in water 35:65
Flow rate: 3
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Retention time: 8 (hydroxyprogesterone caproate)
Internal standard: hydroxyprogesterone caproate

OTHER SUBSTANCES
Simultaneous: levothyroxine

KEY WORDS
tablets; hydroxyprogesterone caproate is IS

REFERENCE
Das Gupta,V.; Odom,C.; Bethea,C.; Plattenburg,J. Effect of excipients on the stability of levothyroxine sodium tablets, *J.Clin.Pharm.Ther.*, **1990**, *15*, 331–336.

SAMPLE
Matrix: ileostomy effluent
Sample preparation: Dilute ileostomy effluent 1:2 by weight with water. Extract 3 g aliquot three times with 10 mL dichloromethane by shaking for 1 min and centrifuging at 2000 rpm for 2 min. Wash combined extracts successively with 2 mL 0.1 M NaOH and 4 mL water by shaking for 30 s and centrifuging for 1 min then dry the organic layer under air at 40°. Take up the extract in 1 mL MeOH, add 1.1 mL water and apply to C18 Bond Elut SPE cartridge. Wash with 10 mL water, wash with 5 mL MeOH:water 45:55, elute with 2 mL MeOH. Add 50 μL 20 μg/mL progesterone to the eluate, dry at 40°, take up in 100 μL MeOH, inject 10 μL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:50 mM pH 3.0 sodium phosphate buffer 55:45
Flow rate: 3
Injection volume: 10
Detector: UV 254 and 238

CHROMATOGRAM
Retention time: 6.0
Internal standard: progesterone (11.6)

OTHER SUBSTANCES
Extracted: beclomethasone alcohol, beclomethasone 17-monopropionate, beclomethasone dipropionate

KEY WORDS
SPE; 17-hydroxyprogesterone is IS

REFERENCE
Levine,D.S.; Raisys,V.A.; Ainardi,V. Coating of oral beclomethasone dipropionate capsules with cellulose acetate phthalate enhances delivery of topically active antiinflammatory drug to the terminal ileum, *Gastroenterology*, **1987**, *92*, 1037–1044.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm RP-18 C18 (Brownlee)
Mobile phase: MeCN:MeOH:water 30:30:40

Injection volume: 20
Detector: UV 254

KEY WORDS
for 17α-hydroxyprogesterone

REFERENCE
Kane,M.P.; Tsuji,K. Radiolytic degradation scheme for ^{60}Co-irradiated corticosteroids, *J.Pharm.Sci.*, **1983**, *72*, 30–35.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: MeOH:THF:water 26:18:56
Column temperature: 48
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 14.6 (17α)

OTHER SUBSTANCES
Simultaneous: cortisone, estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, testosterone, dexamethasone acetate, estradiol, estrone, progesterone

REFERENCE
Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 3–38.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 10 ODS-1
Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: k' 2.175

OTHER SUBSTANCES
Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), progesterone (UV 240), testosterone (UV 240)

REFERENCE
Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

SAMPLE
Matrix: solutions
Sample preparation: Inject 20 μL aliquot of a MeOH solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil 5-ODS
Mobile phase: THF:water 23:77
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: k' 11.86 (11α-hydroxyprogesterone)
Internal standard: methylprednisolone (k' 11.36)

OTHER SUBSTANCES
Simultaneous: metabolites, betamethasone, corticosterone, cortisone, deflazacort, deoxycorticosterone, dexamethasone, fludrocortisone, fludrocortisone acetate, fluorocortisone, fluorocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, methylprednisolone, prednisolone, prednisone, triamcinolone acetonide, triamcinolone

REFERENCE
Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Extraction and high-performance liquid chromatographic separation of deflazacort and its metabolite 21-hydroxydeflazacort. Application to urine samples, *J.Chromatogr.B*, **1994**, *657*, 248–253.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil phenyl
Mobile phase: Gradient. Carbon dioxide:MeOH from 98:2 to 78:22 over 40 min
Column temperature: 50
Flow rate: 2
Detector: UV

CHROMATOGRAM
Retention time: 9.7

OTHER SUBSTANCES
Simultaneous: testosterone, estradiol, norethisterone, hydrocortisone, estriol, other steroids

KEY WORDS
SFC; 200 bar

REFERENCE
Hanson,M. Aspects of retention behaviour of steroids in packed column supercritical fluid chromatography, *Chromatographia*, **1995**, *40*, 58–68.

SAMPLE
Matrix: tissue
Sample preparation: Extract 70-125 mg tissue four times with 5 mL portions of ether:chloroform 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 80 mm long 10 μm octadecylsilane radial compression (Radial-Pak) (Waters)
Mobile phase: Gradient. A was MeOH:water 50:50. B was MeOH. A:B form 100:0 to 70:30 over 20 min, to 0:100 over 20 min

Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 25 (17-hydroxyprogesterone)

OTHER SUBSTANCES
Extracted: androstenedione, deoxycortisol, hydrocortisone, testosterone
Simultaneous: estriol, estradiol, pregnenolone, progesterone, testosterone enanthate, testosterone propionate

KEY WORDS
tumor

REFERENCE
Kessler,M.J. Analysis of steroids from normal and tumor tissue by HPLC, *Clin.Chim.Acta*, **1982**, *125*, 21–30.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 1.5 μg betamethasone + 100 mg K_2HPO_4 + 500 mg anhydrous sodium sulfate + 5 mL diethyl ether, shake mechanically for 10 min, centrifuge at 2500 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:water from 4:96 to 30:70 over 10 min, to 45:55 over 5 min, to 50:50 over 3 min
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 246

CHROMATOGRAM
Retention time: 14.66
Internal standard: betamethasone (12.83)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, cortisone, deoxycorticosterone, hydrocortisone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide

REFERENCE
Park,S.-J.; Kim,Y.-J.; Pyo,H.-S.; Park,J. Analysis of corticosteroids in urine by HPLC and thermospray LC/MS, *J.Anal.Toxicol.*, **1990**, *14*, 102–108.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 μL 5 μg/mL IS in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS
Mobile phase: MeCN:water 32:68
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 19
Internal standard: methylprednisolone (9)

OTHER SUBSTANCES
Simultaneous: triamcinolone, triamcinolone acetonide, prednisolone, corticosterone, hydroxyprogesterone, fluorocortisone acetate, cortisone, hydrocortisone, fluorocortisone, betamethasone, dexamethasone, prednisone

KEY WORDS
SPE also discussed

REFERENCE
Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Solvent and solid-phase extraction of natural and synthetic corticoids in human urine, *J.Chromatogr.B*, **1994**, *652*, 83–89.

Hydroxypropyl methylcellulose

CAS Registry No.: 9004-65-3
Merck Index: 4889

SAMPLE
Matrix: formulations
Sample preparation: Dilute with an equal volume of MeOH:water 40:60, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 300 × 7.8 Ultrahydrogel 250 250 Å cross-linked methacrylate gel (waters)
Mobile phase: MeOH:buffer 20:80 (Buffer was 95 mM boric acid containing 10 mM KCl, 13 μM sodium borate, and 1.5 mM dextrose.) (At the end of each set of analyses rinse instrument and column with 0.5% sodium azide. (Caution! Sodium azide is carcinogenic, highly toxic, and can form explosive heavy metal azides. Do not discharge to the plumbing system!)
Column temperature: 35
Flow rate: 1
Injection volume: 80
Detector: RI

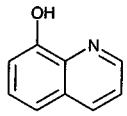
CHROMATOGRAM
Retention time: 10.6

OTHER SUBSTANCES
Simultaneous: PEG 400

KEY WORDS
ophthalmic solutions; SEC

REFERENCE
Delker,G.; Chen,C.; Miller,R.B. Size exclusion chromatographic determination of hydroxypropyl methyl cellulose and polyethylene glycol 400 in an ophthalmic solution, *Chromatographia*, **1995**, *41*, 263–266.

Hydroxyquinoline



Molecular formula: C_9H_7NO
Molecular weight: 145.16
CAS Registry No.: 148-24-3
Merck Index: 4890

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

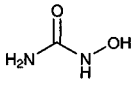
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tol-

metin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Hydroxyurea



Molecular formula: $CH_4N_2O_2$
Molecular weight: 76.06
CAS Registry No.: 127-07-1
Merck Index: 4896

SAMPLE

Matrix: blood
Sample preparation: Mix 500 μL serum, 20 μL 70% perchloric acid, and 20 μL 15.5 mM methylurea, centrifuge at 12000 g for 5 min. Mix 200 μL supernatant with 700 μL BUN acid reagent (No. 535-3, Sigma) and 600 μL BUN color reagent (No. 535-5, Sigma), place the mixture in a boiling water bath to form colored complexes. After 10 min cool the mixture in ice water, inject a 25-100 μL aliquot of the colored solution.

HPLC VARIABLES

Column: 300 × 3.9 Bondclone 10 C18 (Phenomenex)
Mobile phase: MeCN:water 13:87
Flow rate: 1.7
Injection volume: 25-100
Detector: UV 449

CHROMATOGRAM

Retention time: 6.5
Internal standard: methylurea (12.2)

KEY WORDS

serum; derivatization

REFERENCE

Manouilov,K.K.; McGuire,T.R.; Gwilt,P.R. Colorimetric determination of hydroxyurea in human serum using high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *708*, 321–324.

SAMPLE

Matrix: blood
Sample preparation: Vortex plasma with two volumes of 5% trichloroacetic acid for 30 s, centrifuge at 7000 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 3.2 3 μm Phase-II ODS (Bioanalytical Systems)
Mobile phase: 50 mM sodium acetate containing 5 mM tetrabutylammonium hydroxide, adjusted to pH 6.75 ± 0.02 with 50 mM acetic acid
Flow rate: 0.5
Injection volume: 5
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode + 800 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 1.1 (elute for 5 min to remove plasma components)

Limit of detection: 20 μM

OTHER SUBSTANCES
Noninterfering: acetaminophen, caffeine, carbamazepine, ethosuximide, morphine, phenobarbital, phenytoin, salicylic acid, valproic acid

KEY WORDS
plasma

REFERENCE
Havard,J.; Grygiel,J.; Sampson,D. Determination by high-performance liquid chromatography of hydroxyurea in human plasma, *J.Chromatogr.*, **1992**, *584*, 270–274.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 200 μL trichloroacetic acid, vortex for 30 s, centrifuge at 5000 g for 5 min, pass the supernatant through a 1 mL Bond Elut C18 SPE cartridge, inject an aliquot of the eluate.

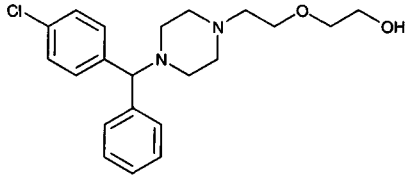
HPLC VARIABLES
Guard column: 20 mm long Supelguard LC 18 (Supelco)
Column: 150 × 4.6 Supelcosil LC 18
Mobile phase: 50 mM Sodium acetate adjusted to pH 6.75 with acetic acid
Flow rate: 0.8
Detector: E, ESA Coulochem II, Model 5020 guard cell 650 mV, Model 5011 analytical cell 600 mV

CHROMATOGRAM
Limit of detection: 600 nM
Limit of quantitation: 1.3 μM

KEY WORDS
pharmacokinetics; serum; SPE

REFERENCE
Villani,P.; Maserati,R.; Regazzi,M.B.; Giacchino,R.; Lori,F. Pharmacokinetics of hydroxyurea in patients infected with human immunodeficiency virus type I, *J.Clin.Pharmacol.*, **1996**, *36*, 117–121.

Hydroxyzine



Molecular formula: $C_{21}H_{27}ClN_2O_2$
Molecular weight: 374.91
CAS Registry No.: 68-88-2, 2192-20-3 (di HCl), 10246-75-0 (pamoate)
Merck Index: 4897
Lednicer No.: 1 59, 4 118

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 1 μg/mL triprolidine + 250 μL 10% KOH + 5 mL ether, vortex, centrifuge. Remove ether layer and add it to 100 μL 0.5% phosphoric acid, vortex, centrifuge, remove most of ether layer and discard it, remove traces of ether by nitrogen at room temperature for 2-3 min, inject all of aqueous layer.

HPLC VARIABLES
Column: Waters CN reverse-phase radial compression

Mobile phase: MeCN:buffer 27:73 (Buffer was 75 mM pH 3.0 phosphate buffer containing 20 mM dibutylamine and 50 ng/mL triprolidine.)
Flow rate: 1
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 6.9
Internal standard: triprolidine (3.6)
Limit of detection: 3 ng/mL

KEY WORDS
serum

REFERENCE
Simons,F.E.R.; Simons,K.J.; Frith,E.M. The pharmacokinetics and antihistaminic of the H_1 receptor antagonist hydroxyzine, *J.Allerg.Clin.Immunol.*, **1984**, *73*, 69–75.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 3 μg/mL IS + 1 mL 1 M pH 5.0 sodium citrate buffer + 3 mL ethyl acetate, vortex 1 min, centrifuge at 4000 rpm for 15 min, remove organic layer, repeat extraction. Combine organic layers and add 200 μL 1.7% phosphoric acid, vortex 1 min, centrifuge 5 min, remove and discard ethyl acetate layer, remove traces of ethyl acetate from aqueous layer using a stream of nitrogen, inject.

HPLC VARIABLES
Column: radial 4 μm NovoPak C18 radial compression
Mobile phase: MeCN:buffer 46:54 (Buffer was 10 mM pH 2.9 KH_2PO_4 + 20 mM sodium 1-decanesulfonate.)
Flow rate: 1.4
Detector: UV 229

CHROMATOGRAM
Retention time: 3.6
Internal standard: P-265, an ethoxy derivative of cetirizine (6.8)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: cetirizine

KEY WORDS
serum

REFERENCE
Simons,K.J.; Watson,W.T.A.; Chen,X.Y.; Simons,F.E.R. Pharmacokinetic and pharmacodynamic studies of the H_1-receptor antagonist hydroxyzine in the elderly, *Clin.Pharmacol.Ther.*, **1989**, *45*, 9–14.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 8.62
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF
Sample preparation: Plasma. Centrifuge blood at 7000 rpm, decant 100 μL plasma. Mix 100 μL plasma with 200 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot. CSF. Add 25 μL water to 25 μL CSF, mix with 50 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: Regis SPS phenyl
Mobile phase: MeCN:pH 3.0 water 21:79 containing 9 mM decanesulfonic acid
Column temperature: 60

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Chou,K.-J.; Donovan,M.D. Distribution of antihistamines into the CSF following intranasal delivery, *Biopharm.Drug Dispos.*, **1997**, *18*, 335–346.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.267

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Prepare solutions in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.0 5 μm Lichrosorb RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 9.8% triethylamine and 0.35% sodium methanesulfonate with pH adjusted to 2.85 with sulfuric acid.)
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 12.3
Limit of detection: 180 ng/mL
Limit of quantitation: 600 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE

Simpson,D.; Skellern,G.G.; Miller,J.H.MB. Method for the control of known impurities in hydroxyzine hydrochloride, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1371–1375.

SAMPLE

Matrix: formulations
Sample preparation: 5 mL Formulation + 5 mL IS solution, make up to 50 mL with MeOH, inject 10 μL aliquot. (IS solution was 0.2 mg p-nitroacetophenone and 2.5 mg isobutyrophenone per mL of MeOH.)

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water:reagent 25:60:15, pH 2.6 (Reagent was MeOH containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate.)
Flow rate: 2
Injection volume: 10
Detector: UV 257

CHROMATOGRAM

Retention time: 9
Internal standard: p-nitroacetophenone (6) and isobutyrophenone (13)

OTHER SUBSTANCES

Simultaneous: benzyl alcohol, benzoic acid, benzaldehyde, p-chlorobenzoic acid, p-chlorobenzaldehyde, p-chlorobenzophenone

KEY WORDS

injections; stability-indicating

REFERENCE

Menon,G.N.; Norris,B.J. Simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol in injection solutions by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 697–698.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, er-

gosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 58:1.5:0.5:40
Flow rate: 1.5
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 12

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 2.42

OTHER SUBSTANCES
Simultaneous: benactyzine, buclizine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 11.40 (A), 6.27 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

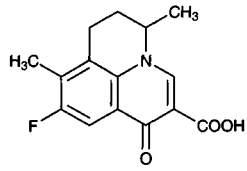
CHROMATOGRAM
Retention time: k' 8.32

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Ibafloxacin



Molecular formula: $C_{15}H_{14}FNO_3$
Molecular weight: 275.28
CAS Registry No.: 91618-36-9
Merck Index: 4919

SAMPLE
Matrix: tissue
Sample preparation: Mix 2 g minced tissue with 4 g anhydrous sodium sulfate until homogenized. Add 10 ml ethyl acetate, shake mechanically for 10 min, centrifuge at 1500 *g* for 10 min. Transfer organic layer into another tube and repeat extraction on the tissue pellet with 10 mL ethyl acetate. Evaporate combined organic phases under a stream of nitrogen at 50°. Dissolve residue in 1 mL MeCN:2.7 mM pH 2.5 oxalic acid 50:50, vortex, sonicate for 5 min, filter through 0.45 μm filter (GHP Acrodisc GF, Gelman Sciences, USA).. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Shandon, UK)